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Proteinic prorenin-releasing-stimulator (PRS) in the rat submandibular gland

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The release of prorenin as well as renin from rat renal slices was confirmed by a rat prorenin-prosegment ELISA system and an assay system for determining the renin activity. A significant increase of the prorenin release was found by adding rat submandibular gland extract to the slice medium, indicating the existence of a prorenin-releasing stimulator (PRS) in the extract. The p*I* and molecular mass of PRS were 8.5–8.7 and 28–30 kDa, respectively. The PRS was completely inactivated by boiling or a proteinase treatment.

Renin releasing stimulator, pro-; Cortical slice; Submandibular gland; ELISA, prorenin-prosegment; (Rat kidney)

1. INTRODUCTION

Renin (EC 3.4.23.15) is a key enzyme in the renin-angiotensin system which plays an important role in the control of blood pressure and the electrolyte balance [1,2]. Renin is synthesized as preprorenin, which is processed to prorenin (trypsin activatable inactive renin), and then to active renin [3–6]. A large amount of inactive renin was observed in the circulation [2,7,8], the amount of which was 5–10 times more than that of active renin, and was identified as prorenin [6,9,10].

Little information is currently available for the source and activation mechanism of prorenin in the circulation, although prorenin has attracted the attention of a very large number of investigators (review [11,12]). During our studying plasma prorenin in rats, the prorenin level was observed to decrease after sialo-adenectomy [13]. In this study, we found proteinic prorenin-releasing stimulator (PRS) in the rat SMG using renal slices and a prorenin-prosegment ELISA.

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Abbreviations: ELISA, enzyme-linked immunosorbent assay; IEF, isoelectric focusing; PRS, prorenin-releasing stimulator; Ang, angiotensin; SMG, submandibular gland

2. MATERIALS AND METHODS

SMG extract was prepared by homogenizing rat SMG with 10 vols of 0.02 M Tris-HCl, pH 8.4, containing 1 mM diisopropylfluorophosphate, 10 mM EDTA, 10 mM *n*-ethylmaleimide.

PRS activity was determined as the increment of prorenin release from 1 g of cortical slices of rat kidneys into a modified Earle's medium (DIFCO Lab., USA), pH 7.4, at 37°C for 1 h after adding crude PRS preparations. The kidney cortical slices were prepared from male Wistar rats weighing 250–300 g under anesthesia of sodium pentobarbital (35 mg/kg, i.p.). The kidneys were quickly removed, decapsulated, immersed in the cold medium, cut with a microslicer (DTK-100, Dosaka EM., Japan) into slices of 0.5 mm thickness, and medulla was removed from the slices. Each of 3 cortical slices (100–200 mg) was preincubated in a flask containing 3 ml of the medium for 30 min at 37°C in an atmosphere of 5% CO_2/95% O_2, washed 3 times, and incubated for 1 h with 3 ml of the medium containing 100 μl of SMG extract or crude PRS preparation. Each flask was maintained under a hood filled with the gas throughout the

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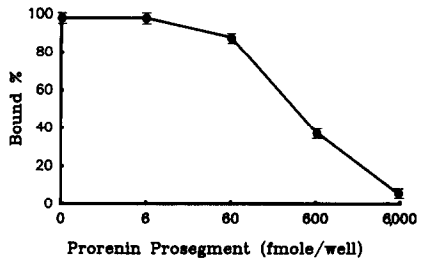

Fig.1. A typical standard curve for rat prorenin prosegment by the present ELISA. Absorbance of 100% binding was 0.783 ± 0.040 at 450 nm (n = 12).

experiment. As a control experiment, 100 μl of the solvent for PRS instead of a PRS preparation was added into the medium. Each incubated medium was stored at −20°C until analysis.

The amount of prorenin was determined by (i) the renin assay system including Ang I RIA [14] after activation of prorenin by trypsin [13] and/or by (ii) the prorenin-prosegment ELISA system as described as follows. The prosegment antibodies (100 μl, 1:3000 dilution), which were raised against the rat prorenin prosegment (48–62, GVDMTRISAEYGEFI) (Uchiyama et al., unpublished data), were adsorbed in the 96 wells of a microplate (Greiner). Each well was incubated for 1 h at 37°C with 100 μl of the incubated media or various amounts of the authentic prosegment (48–62, Peptide Inst. Inc., Japan) and 100 μl of the prosegment-peroxidase conjugate obtained by glutaraldehyde treatment, washed 5 times with 300 μl of phosphate-buffered saline containing 0.05% Tween 20, and then incubated with 150 μl of 55 mM 3,3′,5,5′-tetramethylbenzidine for 5 min at 37°C. The peroxidase reaction was started by adding 50 μl of H_2O_2, performed for 15 min, and stopped with 100 μl of 2 M H_2SO_4. The products were measured at 450 nm by a microplate reader (MPR-A4, Tosoh, Japan). The prorenin prosegment from 6 fmol to 6 pmol was detectable by this ELISA system (fig.1). Active renin did not interfere with the assay system. The molar concentration of the prorenin prosegment was assumed to be that of prorenin.

3. RESULTS AND DISCUSSION

Trypsin-activatable renin besides active renin was released from the rat kidney cortical slices. These amounts were 1.9 ± 0.2 and 6.2 ± 0.3 μg

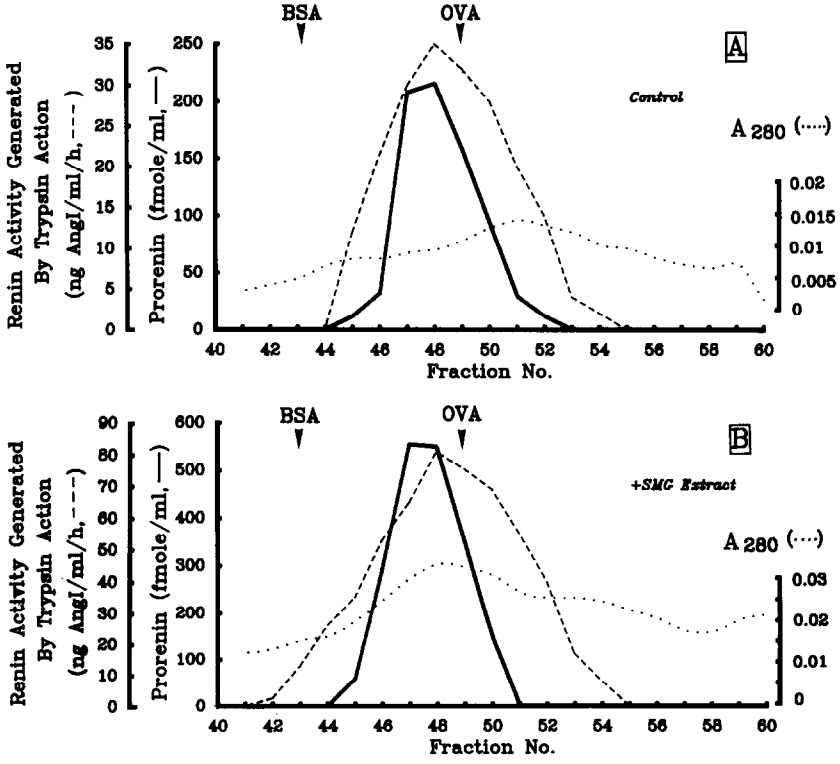

Fig.2. Gel filtration of prorenin released into slice media. Aliquots of each slice medium (100 μl), obtained in the absence (A) or presence (B) of the SMG extract, were applied on a TSK gel G2000SW column (7.5 × 600 mm, Tosoh, Japan) at 1 ml/min of the flow rate. Each 300 μl fraction was collected. Prorenin was assayed in each fraction by the prosegment ELISA (——) and trypsin activation method (- - -). BSA, bovine serum albumin; OVA, ovalbumin.

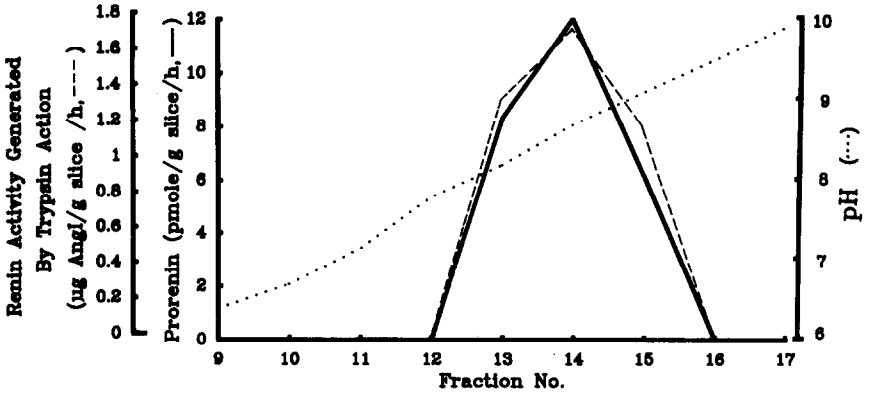

Fig.3. IEF of PRS in the SMG extract. A preparative IEF for 40 ml of the SMG extract was achieved in the presence of 2% Bio-Lyte (pH 3–10) at 12 W for 3 h at 4°C using Rotofor (BioRad). Each fraction (2.5 ml) from pH 4.0 to 10 was assayed for PRS activity.

Ang I/g slices/h (n = 5), respectively. These results confirmed observations of the trypsin-activatable renin release from kidney cortical slices in hog [15] and rabbit [16]. The level of the trypsin-activatable renin release increased up to 5.7 ± 0.3 μg Ang I/g slices/h (n = 5) after adding the SMG extract, whereas the extract had no effect for the active renin release. 100 μl of each medium, which were obtained by incubating the cortical slices with or without SMG extract, was applied to a TSK gel G200SW column. The trypsin-activatable renin was eluted at the same region (45 kDa) as prorenin detected by the prorenin-prosegment ELISA, and the amount of the prorenin release augmented approx. 3 times after adding the SMG extract (fig.2), indicating that the trypsin-activatable renin was identical with prorenin. The SMG extract did not contain prorenin. From these results, we concluded that a prorenin-releasing stimulator (PRS) exists in rat SMG.

The pI and molecular mass of PRS were estimated at 8.5–8.7 (fig.3) and 28–30 kDa (fig.4) according to IEF of SMG extract and gel filtration of PRS fraction obtained by the IEF, respectively. The PRA fraction isolated by the IEF and gel filtration was completely inactivated after boiling for 3 min or incubating with 0.5 mg/ml of trypsin at 20°C for 6 h, indicating that PRS should be proteinic. PRS with these properties is not the same as any other substance found so far in SMG [17]. This is the first paper reporting a proteinic PRS in SMG, although verapamil, a calcium-channel blocker, was reported to stimulate specifically prorenin release from rabbit cortical slices [16]. Fur-

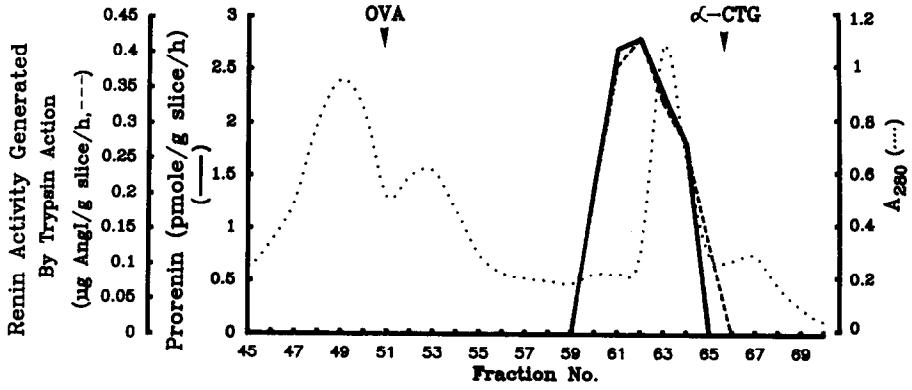

Fig.4. Gel filtration of PRS obtained by the IEF of the SMG extract. Aliquots of fraction no.14 shown in fig.3 (100 μl) were chromatographed under the same conditions as described in the legend for fig.2. Each fraction was assayed for the PRS activity. α-CTG, α-chymotrypsinogen.

ther biochemical and physiological studies of PRS will provide a clue to why such a large amount of prorenin is secreted during in vivo circulation.

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